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A NEW CLASS OF ANTIMALARIAL AGENTS: 2-ACETYLPYRIDINE THIOSEMICA--ETC(U)
JUN 78 J P SCOVILL, J F BARTOSEVICH

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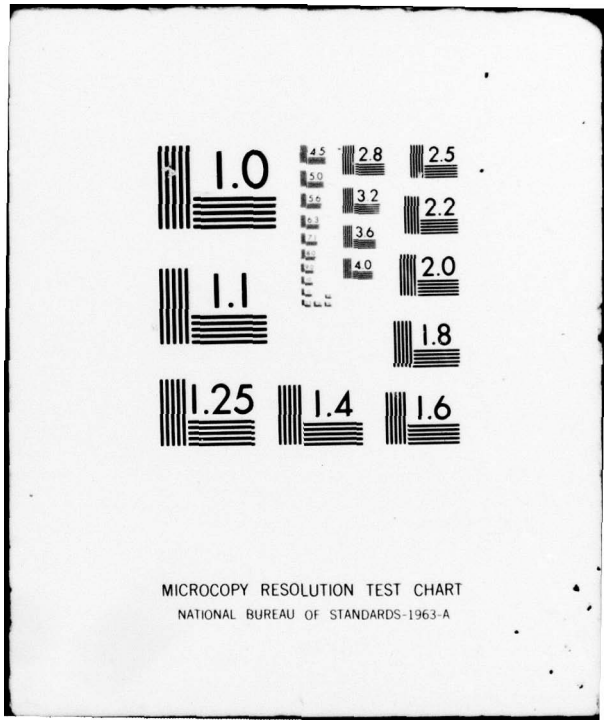
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6 A NEW CLASS OF ANTIMALARIAL AGENTS: 2-ACETYLPYRIDINE THIOSEMICARBAZONES (U)

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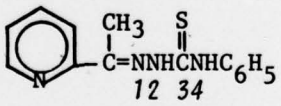
Malaria remains a formidable global problem in that more than a billion people live in malarious regions and as many as 400 million live in areas where eradication programs have not as yet begun. The continued capability of malaria to disrupt military operations was most recently demonstrated during the Vietnam conflict. The problem has been further complicated by the emergence of highly drug-resistant strains of Plasmodium falciparum--a phenomenon which is being seen increasingly in many other parts of the world. Thus the development of new antimalarial drugs continues to be a high priority project in the Walter Reed Army Institute of Research.

Our investigations into the area of thiosemicarbazone chemistry was prompted by the discovery of the antimalarial activity of 2-acetylpyridine 4-phenyl-3-thiosemicarbazone (WR 190,598). We began a



systematic program of structural modification of this parent compound in an effort to elucidate the molecular features essential for activity.

Examination of the structure of WR 190,598 suggested a myriad of possible variations. 1) The portion of the molecule derived from 2-acetylpyridine might be replaced by any of a large number of aromatic, heteroaromatic, cycloaliphatic aldehydes or ketones. 2) As for the

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thiosemicarbazone portion of the molecule, substitution is possible on the nitrogen atom at position 2, while at position 4 a virtually limitless number of mono- and disubstituted analogs can be conceived. 3) The sulfur-containing function at position 3 can be replaced by an oxygen-containing carbonyl group.

The screening of candidate drugs was performed in mice at the University of Miami under a U.S. Army contract.

Results

Initially, a series of 4-substituted thiosemicarbazones was synthesized in which the pyridylethylidene moiety derived from 2-acetylpyridine was replaced by a variety of heterocyclic aldehydes, such as 5-nitrofurfural, 2-thiophenecarboxyaldehyde, and indole-3-carboxyaldehyde; and phenyl ketones and aldehydes, such as p-chloro-acetophenone, m-fluorobenzaldehyde, piperonal, 3,4-dimethoxy benzaldehyde, and cinnamaldehyde. 1-Adamantyl methyl ketone was also tried. In no instance was antimalarial activity observed in the above classes of compounds.

Upon returning to the acetylpyridines, it was found that activity was imparted only by the compound bearing the keto function in the 2-position (Cf. Table 1).

Table 1. Antimalarial Activities of Four Isosteres of WR 190,598 and a 2-Substituted Analog.

$$R\text{-}\overset{CH_3}{\overset{|}{C}}\text{=}NNR'C(=X)NHC_6H_5$$

Cmpd No.	R	R'	X	Antimalarial Activity*
WR 190,598	2-Pyr	H	S	C(2/5) 320 mg/kg; C(4/5) 640 mg/kg
WR 232,145	2-Pyr	CH_3	S	Active 320 mg/kg; C(1/5) 640 mg/kg
WR 190,429	3-Pyr	H	S	Inactive
WR 190,612	4-Pyr	H	S	Inactive
WR 210,219	Ph	H	S	Inactive
WR 233,150	2-Pyr	H	O	Inactive

Pyr = pyridine; Ph = phenyl
*C = cures; number of treated mice which survived >60 days.
Active = at least a 100% increase in the mean survival time of treated mice over that of controls.

These data, along with others, led us to believe that the presence of a 2-pyridyl ring at position R is essential, as is the presence of sulfur in the carbonyl group. The greatly diminished activity of WR 232,145 suggested that preparation of 2-substituted derivatives would not be rewarding. Thus our consideration was limited to the study of the effects of replacement of the methyl group in 2-acetylpyridine and effects of mono- and disubstitution of the nitrogen at position 4.

Many ring substituted 4-phenyl-3-thiosemicarbazones were prepared which contained alkyl, hydroxyl, nitro or halo substituents attached to the phenyl ring. Some, such as the 2-fluorophenyl and 3-fluorophenyl analogs, produced cures, but none showed any substantial improvement over the unsubstituted parent compound, WR 190,598.

Substitution of 2-formyl or 2-propionylpyridine for 2-acetylpyridine resulted in a reduction or abolition of activity (Table 2). Thiosemicarbazones containing side chains derived from useful antimalarial drugs were also prepared. Thus the 4-amino-3-diethylamino-o-cresol group from amodiaquine was attached to position 4 to give WR 229,090 as was the 4-diethylamino-1-methylbutyl side chain from chloroquine and pamaquine, producing WR 224,895. Both of these compounds were inactive.

Table 2. Antimalarial Activity of Some Substituted 4-Phenyl Analogs of 2-Acetylpyridine

2-Pyr(R)-C=NNHC(=S)NHR'

Cmpd No.	R	R'	Antimalarial Activity*
WR 32,974	H	C_6H_5	Inactive
WR 190,594	CH_3	C_6H_5	C(2/5) 320 mg/kg; C(4/5) 640 mg/kg
WR 228,397	CH_3	$2\text{-}ClC_6H_4$	Active 320 mg/kg; Active 640 mg/kg
WR 229,090	CH_3	$4\text{-}OH\text{-}3\text{-}CH_2N(C_2H_5)_2C_6H_3$	Inactive
WR 224,895	CH_3	$4\text{-}CH(CH_3)(CH_2)_3N(C_2H_5)_2$	Inactive
WR 227,502	CH_2CH_3	$2\text{-}ClC_6H_4$	Inactive

*See Table 1 for explanation of biological data

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A large number of 4-alkyl and 4-cycloalkyl thiosemicarbazones were prepared of which the 4-benzyl and 4-phenethyl thiosemicarbazones showed some activity. The 4-alkyl compounds, however, were inactive. The medicinally interesting admantyl group when attached in the 4 position gave an active compound (Table 3). This suggested that favorable results might be obtained by the attachment of bulky

Table 3. Antimalarial Activities of Some 4-Alkyl and 4-Cycloalkyl Thiosemicarbazones

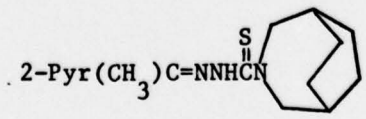
$2\text{-Pyr}(CH_3)C{=}NNHC(=S)NHR$

Cmpd No.	R	Antimalarial Activity*
WR 204,584	adamantyl	Active 320 mg/kg, C(1/5) 640 mg/kg
WR 229,805	$-CH\overset{\frown}{(}CH_2)_5$	C(2/5) 160 mg/kg, C(3/5) 320 mg/kg C(2/5) 640 mg/kg, T(3/5) 640 mg/kg**
WR 231,011	$-CH\overset{\frown}{(}CH_2)_7$	Active 640 mg/kg, T(1/5)**
WR 231,534	$-C(CH_3)_2CH_2C(CH_3)_3$	Active 640 mg/kg
WR 227,817	$-CH_2C_6H_5$	Active 320 mg/kg, C(3/5) 640 mg/kg

* See Table 1 for explanation of biological data
**Toxic deaths

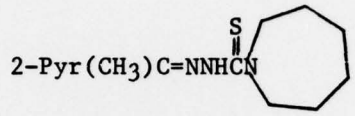
groups at this location. To this end a number of derivatives incorporating features of the adamantyl group were prepared. These included the 4-t-butyl and 4-t-octyl thiosemicarbazones, the latter possessing marginal activity (Table 3). However, simplification of the adamantyl ring system gives cyclohexane and 2-acetylpyridine 4-cyclohexyl thiosemicarbazone possessed appreciable antimalarial activity.

A fanciful connection between the adamantyl ring system and that of 3-azabicyclo[3.2.2]nonane suggested the preparation of WR 230,190.



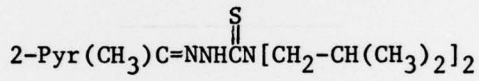
WR 230,190

This compound possesses excellent antimalarial activity, producing cures at a dosage as low as 20 mg/kg. The ED_{50} was estimated to be 50 mg/kg. Simplification of structure of WR 230,190 by elimination of the two carbon bridge gives WR 231,010, which also exhibited good



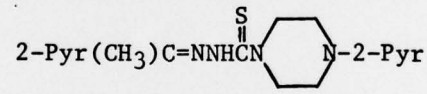
WR 231,010

antimalarial activity. Scission of the carbon bridges of 230,190 yields WR 232,178, which was inactive. Many cyclic amines gave deriv-



WR 232,178

atives with good antimalarial activity (Table 4). Exploitation of this lead led to the synthesis of WR 235,591, which is the most active



WR 235,591

antimalarial yet produced in this class of compounds.

Consideration of the tuberculostatic property of certain pyridylcarboxaldehyde thiosemicarbazones suggested that antimalarial 2-acetylpyridine thiosemicarbazones might possess useful antibacterial activity. We were able to secure the cooperation of Dr. Arthur Dobek of the Division of Communicable Diseases and Immunology (WRAIR), who established an antibacterial screening system. These compounds were ineffective against Gram negative bacteria, however, they possessed high activity against Gram positive organisms (Table 5). The minimal inhibitory concentration (MIC) of WR 230,190 against 36 recent clinical isolates of Staphylococcus aureus was found to be 1 µg/ml. Further testing of 12 of the testing strains showed that this concentration was also bacteriocidal. Some compounds which are devoid of antimalarial activity are highly bacteriostatic.

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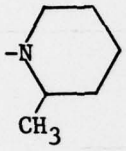
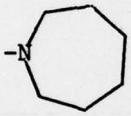
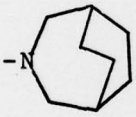
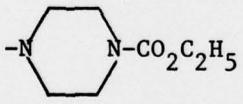
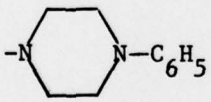
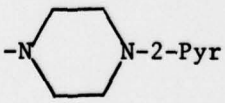
Table 4. Antimalarial Activities of Some 4,4-Disubstituted Thiosemicarbazones

2-Pyr(CH_3)C=NNHC(=S)R

Cmpd No.	R	Antimalarial Activity*
WR 231,533		C(2/5) 40 mg/kg
WR 231,010		C(3/5) 40 mg/kg; C(4/5) 80 mg/kg
WR 230,190		C(1/5) 20 mg/kg; C(3/5) 40 mg/kg; C(5/5) 160 mg/kg
WR 233,545		C(2/5) 40 mg/kg
WR 232,706		C(4/5) 160 mg/kg
WR 235,591		C(3/5) 20 mg/kg; C(4/5) 40 mg/kg; C(5/5) 80 mg/kg

*See Table 1 for an explanation of biological data.

Table 5. Antibacterial Activities* of Some Selected 2-Acetylpyridine Derivatives

2-Pyr(CH_3)C=NNHC(=S)-R

Walter Reed Code Number	WR 223,662	WR 229,805	WR 230,190	WR 232,704
	4-ClC_6H_4-NH	Cyclohexyl-NH	3-(3-azabicyclo [3.2.2]nonyl)	1-(4-methyl-piperidyl)
Bacterial Strain		1	<0.25	
Staph. aureus 15A4 (16 Feb 1944)	3	1	<0.25	
15A5 (1 Jul 1944)	3	1	<0.25	
ATCC 10537 (9 Nov 1949)	1	1	<0.25	
Lafferty	1		<0.25	2
Strep. durans ATCC 9810 (27 Jul 1955)	2		<0.25	2
Strep. pyrogenes 12A2 (22 Feb 1944)	1		<0.25	0.5
ATCC 10100 (8 Aug 1947)	0.25			0.25
Group D Enterococcus**		4		4
Group C Meningococcus**		0.25		<0.25

* Minimal Inhibitory Concentrations given in µg/ml.
**Recent clinical isolates.

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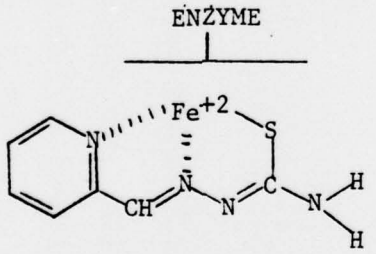
A Possible Mechanism of Action:

The biological basis of the antibacterial and antimalarial activity of the compounds described in this paper has not been studied as yet. However, French and coworkers [J. Med. Chem., 13, 1117 (1970)] have proposed a provisional explanation of the antitumor effects of the generic class to which these compounds belong. The following observations may be pertinent: 1) Thiosemicarbazones of this class are strong chelating agents and act as tridentate ligands for suitable metal ions, i.e., Fe, Cu, Zn. 2) Iron stimulates the activity of cytidine diphosphate reductase isolated from Novikoff hepatoma. 3) Ribonucleoside reductases (RDR) isolated from various mammalian tumors have an obligatory requirement for iron. 4) 1-Formylisoquinoline thiosemicarbazone is a very potent inhibitor of DNA synthesis but has little effect upon RNA and protein synthesis by sarcoma 180 cells in vivo. 5) The better tumor inhibitors had $K_i 50$ against a mammalian RDR in the range of 10^{-6} to 10^{-7}M. Related compounds such as 3-formylpyridine thiosemicarbazone which did not contain the proper tridentate ligand orientation were only active at much higher concentrations (10^{-3}M) and were not active against the tumor in vivo.

In view of these observations, French has proposed that heterocyclic formylthiosemicarbazones act by forming a complex with enzyme-bound iron as illustrated below:



Tenacious binding of the enzymatically-essential iron by the thiosemicarbazone would thus block the active site of the enzyme. This concept also offers an explanation for the lack of antimalarial activities of the isosteres of WR 190,598 (see Table 1); that is, those compounds in which the orientation of nitrogen in the pyridyl ring precludes chelation should be inactive and the compound in which sulfur has been exchanged for the non-chelating oxygen function should also be inactive.

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Organic Chemistry

Three synthetic procedures proved to be useful for the preparation of the thiosemicarbazones required for this study.

In Scheme A, a primary amine was converted to the corresponding isothiocyanate (1), ordinarily by employing thiophosgene. Reaction of 1 with hydrazine afforded a thiosemicarbazide 2. Condensation of this intermediate with 2-acetylpyridine provided the 4-monosubstituted thiosemicarbazone 3. A drawback of this approach is that only thiosemicarbazones monosubstituted at position 4 can be prepared in this manner,

Scheme A

$$RNH_2 + C(=S)Cl_2 \longrightarrow \underset{1}{R\text{-}N=C=S} \xrightarrow{H_2NNH_2} \underset{2}{R\text{-}NHC(=S)NHNH_2}$$

$$\xrightarrow{2\text{-}Pyr(CH_3)C=O} 2\text{-}Pyr(CH_3)C=NNHC(=S)NHR$$

$$\underset{3}{}$$

as 2-acetylpyridine proved to be usually resistant to condensation with 2,4-disubstituted thiosemicarbazides.

In Scheme B, reaction of hydrazine and carbon disulfide in the presence of sodium hydroxide yielded a dithiocarbazate. Alkylation of this dithiocarbazate with either iodomethane or dimethyl sulfate gave methyl dithiocarbazate (4). Condensation of 4 with 2-acetylpyridine gave the versatile intermediate, 3-[1-(2-pyridyl)ethylidene]-S-methyldithiocarbazate, 5. Reaction of 5 with primary amines gave 4-monosubstituted thiosemicarbazones such as 3 while secondary amines or cyclic amines produced 4,4-disubstituted thiosemicarbazones, 6. In addition, reaction of 5 was not limited to more active nucleophiles, as excellent yields could be obtained with many primary aromatic amines. However, 5 was resistant to reaction with secondary aromatic amines, such as N-methylaniline.

Scheme B

$$\underset{4}{H_2NNHC(=S)SCH_3} + 2\text{-}Pyr(CH_3)C=O \longrightarrow \underset{5}{2\text{-}Pyr(CH_3)C=NNHC(=S)SCH_3} \xrightarrow{HNR'R''}$$

$$\longrightarrow 2\text{-}Pyr(CH_3)C=NNHC(=S)NR'R''$$

$$6,\ 3(R'=H)$$

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Scheme C involved the reaction of 2-acetylpyridine with hydrazine to yield the hydrazone 7. Reaction of this hydrazone with an isothiocyanate 1 produced a 4-monosubstituted thiosemicarbazone 3. This reaction was especially useful when the required isothiocyanate was commercially available.

Scheme C

$$2\text{-Pyr}(CH_3)C{=}O + H_2NNH_2 \longrightarrow \underset{7}{2\text{-Pyr}(CH_3)C{=}NNH_2} \xrightarrow{1} 3$$

Conclusion

The preparation of WR 231,010 represents an 8-fold improvement in efficacy over that of our initial model, WR 190,598. Now that a clearer understanding of the relationship between structure and activity in this new class of antimalarial agents has been obtained, it is hoped that continued efforts in this area will produce even more fruitful results.

Acknowledgement

We thank Colonel Craig J. Canfield and Dr. Thomas R. Sweeney for interest and encouragement throughout these studies.